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Phenolic Constituents and Their Anti-inflammatory Activity from *Echinochloa utilis* Grains

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Abstract – Seven phenolic compounds including *p*-coumaric acid (**1**), 4-hydroxybenzoic acid (**2**), 4-hydroxybenzaldehyde (**3**), vanillic acid (**4**), luteolin (**5**), acacetin (**6**), and tricin (**7**), were isolated from the methylene chloride and ethyl acetate fractions of *Echinochloa utilis* grains. Compounds (**1** - **4**, **6**) were isolated for the first time from this plant. These compounds were tested for inhibitory activities against LPS-induced NO production in RAW 264.7 cells. Compounds **5** and **6** displayed significant inhibitory effects, with IC_{50} values of 27.9 ± 2.6 and 14.0 ± 1.1 μM, respectively. The results suggested that *E. utilis* ethanolic extract may be used as a potential source of anti-inflammatory agents and functional foods for the treatment of allergic diseases.
Keywords – *Echinochloa utilis*, Phenolic compounds, NO production

Introduction

Inflammation is a normal physiological and immune response to injury that occurs in response to trauma, infection, autoimmune or microbial activities, heat and toxins.[1,2] Nitric oxide (NO) plays a wide variety of roles in biological processes and it is recognized as a mediator and regulator of the inflammatory response.[3] In the lipopolysaccharide (LPS)-stimulated macrophages, NO is generated by the process of single enzymes such as inducible NOS (iNOS) and cyclooxygenase-2 (COX-2).[4]

Echinochloa utilis (barnyard millet, Japanese barnyard millet, *E. esculenta*, *E. frumentacea*) is a crop species belonging to the Poaceae family and is cultivated in Korea, Japan, and China.[5] The grains of this plant have excellent agricultural characteristics, including animal forage, easy cultivation, salt tolerance, drought resistance, cold tolerance, and assured crop harvest.[6,7] In several Asian countries, *E. utilis* grains are consumed in a manner similar to rice. Furthermore, they are used as a functional food to treat allergic diseases such as atopic dermatitis.[8,9]

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A previous biological study on *E. utilis* grains revealed its strong antioxidant activity.[9] However, information on the anti-inflammatory activity of this plant is lacking. Therefore, we conducted this study to isolate and determine their anti-inflammatory activity from this plant. We describe the isolation, structural determination and NO production inhibitory activity in LPS-stimulated RAW246.7 cells for the components isolated from *E. utilis* grains.

Experimental

General experimental procedures – The infrared spectroscopy (IR) spectra were measured on a Mattson Polaris FT/IR-300E spectrophotometer. The nuclear magnetic resonance (NMR) spectra were recorded in methanol-d_4 on Varian OXFORD-AS 400 MHz instrument (Palo Alto, CA, USA) or Agilent 600 MHz instrument (Santa Clara, CA, USA). Low resolution FAB-MS and EI-MS data were measured on a Quattro II mass spectrometer. Open column chromatography was performed on silica gel (Merck, 63 - 200 μm particle size), RP-18 (Merck, 150 μm particle size), and Sephadex LH-20 (Pharmacia Co. Ltd.). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in ethanol, followed by heating. For thin-layer chromatography, pre-

coated TLC was carried out on silica gel 60 F_{254} and RP-18 plates from Merck. High performance liquid chromatography (HPLC) system was carried out using a Gilson system with an UV detector and an Optima Pak C18 column (10 × 250 mm, 10 μm particle size, RS Tech Corp., Korea).

Plant materials – The grains of *E. utilis* were collected from National Institute of Crop Science of Miryang in February 2013. A voucher specimen (EU.2013002) has been deposited at the College of Pharmacy, Catholic University of Daegu.

Extraction and isolation – The dried grains of *E. utilis* (30 kg) were extracted with 80% ethanol (20 L × 4 times) at 80 °C for 4 h to yield 1.1 kg of extract. The concentrated extract was suspended in H_2O (6 L), and the resulting H_2O layer was partitioned between H_2O and *n*-hexane (6 L × 7 times, 305 g), CH_2Cl_2 (6 L × 7 times, 68 g), ethyl acetate (EtOAc) (6 L × 7 times, 35 g), *n*-butanol (*n*-BuOH) (6 L × 7 times, 130 g), and H_2O-soluble fractions, respectively. The *n*-hexane, the CH_2Cl_2, the EtOAc, the *n*-BuOH and the H_2O-soluble layers were analyzed by the TLCs, the results showed both the CH_2Cl_2 and EtOAc fractions to be quite similarity. Therefore, they were combined for isolation. The CH_2Cl_2 and EtOAc fractions were subjected to open column chromatography, eluted with gradient solvent system as *n*-hexane-EtOAc-MeOH (1:0:0 to 1:1:0 and 1:1:0.1 to 1:1:1, each 5 L) to obtain 14 fractions (A to N) according to their TLC patterns. These fractions were assayed for the NO production. Fractions G, H, and L displayed strong anti-inflammatory activity. Fraction G (2.8 g) was loaded onto a RP-18 column eluting with a stepwise gradient of MeOH-H_2O (1:1, 2:1, 4:1, 8:1, 12:1) to afford 14 sub-fractions (G-1 to G-14). Sub-fraction G-1 (67.5 mg) was subjected to semi-preparative Gilson HPLC eluted with solvent system of 33% MeOH in H_2O for 60 min to give compounds **2** (7.1 mg, t_R = 38.2 min) and **4** (11.2 mg, t_R = 51.7 min), respectively. Fraction L (1.8 g) was subjected to chromatography over Sephadex LH-20 with MeOH, to afford six sub-fractions (L-1 to L-6). Sub-fraction L-3 (215 mg) was further purified to RP-18 column chromatography, eluted with MeOH-H_2O (1:1, 2:1, 4:1, 8:1 and 12:1), to afford compounds **5** (6.8 mg) and **6** (5.0 mg), respectively. Sub-fraction L-6 (210 mg) was purified by using Sephadex LH-20 eluted with MeOH to yield compound **7** (80.0 mg). Fraction H (2.4 g) was subjected to chromatography over Sephadex LH-20 with methanol, to afford five sub-fractions (H-1 to H-5). Sub-fraction H-4 (326 mg) was further subjected to RP-18 column chromatography, eluted with MeOH-H_2O (1:1, 2:1, 4:1, 8:1 and 12:1), to afford six fractions (H-4-1 to H-4-6). Sub-fraction H-4-3 (160 mg) was subjected to RP-18 column chromatography, eluted with 66% MeOH in H_2O, to afford compounds **1** (40.1 mg) and **3** (8.1 mg), respectively.

***p*-Coumaric acid (1)** – White powder; IR (KBr) cm^{-1}: 3380, 2908, 1672, 1626, 1600, 1520, 1448, 1250, 972, 830, 515; ESI-MS *m/z* 164 $[M]^+$; ^1H NMR (CD_3OD, 600 MHz) δ: 7.58 (1H, d, *J* = 15.9 Hz, H-7), 6.26 (1H, d, *J* = 15.9 Hz, H-8), 7.42 (2H, d, *J* = 8.5 Hz, H-2, 6), 6.79 (2H, d, *J* = 8.5 Hz, H-3, 5); ^{13}C NMR (CD_3OD, 150 MHz) δ: 127.4 (C-1), 131.2 (C-2, 6), 116.9 (C-3, 5), 161.2 (C-4), 146.7 (C-7), 115.9 (C-8) 171.2 (COOH).

4-Hydroxybenzoic acid (2) – White crystalline solid; IR (KBr) cm^{-1}: 3401, 2918, 2800, 2105, 1672, 1600, 852; EI-MS *m/z* 138 $[M]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.86 (2H, d, *J* = 8.6 Hz, H-2, 6), 6.79 (2H, d, *J* = 8.6 Hz, H-3, 5); ^{13}C NMR (CD_3OD, 100 MHz) δ: 170.5 (COOH), 123.2 (C-1), 133.4 (C-2, 6), 116.4 (C-3, 5), 163.7 (C-4).

4-Hydroxybenzaldehyde (3) – White solid; IR (KBr) cm^{-1}: 3409, 1681, 1597, 1509, 1248, 821; FAB-MS *m/z* 123 $[M + H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.76 (2H, d, *J* = 8.7 Hz, H-2, 6), 6.90 (2H, d, *J* = 8.7 Hz, H-3, 5), 9.75 (1H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 193.0 (CHO), 130.4 (C-1), 133.6 (C-2, 6), 117.0 (C-3, 5), 165.3 (C-4).

Vanillic acid (4) – Colorless gum; IR (KBr) cm^{-1}: 3384, 2910, 1672, 1627, 1602, 1512, 1450, 1245, 977, 833, 516; FAB-MS *m/z* 169 $[M+H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.54 (2H, overlapped, H-2, 6), 6.82 (1H, d, *J* = 6.8 Hz, H-3), 3.87 (3H, s); ^{13}C NMR (CD_3OD, 100 MHz) δ: 170.0 (COOH), 124.5 (C-1), 125.1 (C-2), 113.6 (C-3), 152.4 (C-4), 148.5 (C-5), 115.6 (C-6), 56.2 (OCH_3).

Luteolin (5) – Yellow powder; IR (KBr) cm^{-1}: 3421, 1670, 1619, 1621, 1420, 1108, 967, 836; EI-MS *m/z* 287 $[M+H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.38 (2H, overlapped, H-2', 6'), 6.91 (1H, d, *J* = 8.9 Hz, H-5'), 6.54 (1H, s, H-3), 6.44 (1H, d, *J* = 2.1 Hz, H-8), 6.21 (1H, d, *J* = 2.1 Hz, H-6); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.1 (C-2), 104.0 (C-3), 184.0 (C-4), 159.5 (C-5), 100.2 (C-6), 166.5 (C-7), 95.1 (C-8), 163.3 (C-9), 105.4 (C-10), 120.4 (C-1'), 114.3 (C-2'), 147.2 (C-3'), 151.1 (C-4'), 116.9 (C-5'), 123.8 (C-6').

Acacetin (6) – Pale yellow powder; IR (KBr) cm^{-1}: 3172, 2945, 2251, 2126, 1660, 1615; EI-MS *m/z* 285 $[M+H]^+$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.74 (2H, d, *J* = 8.0 Hz, H-2', 6'), 7.38 (2H, d, *J* = 8.0 Hz, H-3', 5'), 6.81 (1H, d, *J* = 2.0 Hz, H-8), 6.49 (1H, d, *J* = 2.0 Hz, H-6), 6.34 (1H, s, H-3), 3.85 (3H, s, OCH_3); ^{13}C NMR (CD_3OD, 100 MHz) δ: 165.8 (C-2), 104.3 (C-3), 183.8 (C-4), 163.1 (C-5), 103.9 (C-6), 166.6 (C-7), 95.2 (C-8), 159.6 (C-9), 105.4

(C-10), 121.9 (C-1'), 129.6 (C-2', 6'), 117.2 (C-3', 5'), 161.0 (C-4'), 56.8 (OCH_3).

Tricin (7) – Yellow powder; IR (KBr) cm^{-1}: 3238, 2930, 2812, 2359, 1661, 1610, 1506, 1480, 1020, 838; EI-MS *m/z* 329 $[M–H]^-$; ^1H NMR (CD_3OD, 400 MHz) δ: 7.16 (2H, s, H-2', 6'), 6.39 (1H, d, J = 2.0 Hz, H-8), 6.12 (1H, d, J = 2.0 Hz, H-6), 6.57 (1H, s, H-3), 3.86 (6H, s, $2OCH_3$); ^{13}C NMR (CD_3OD, 100 MHz) δ: 166.2 (C-2), 104.7 (C-3), 184.0 (C-4), 159.6 (C-5), 100.3 (C-6), 163.4 (C-7), 95.3 (C-8), 166.2 (C-9), 105.4 (C-10), 122.8 (C-1'), 105.5 (C-2',6'), 149.8 (C-3',5'), 141.3 (C-4'), 57.2 (2 × OCH_3).

Cell culture – The RAW 264.7 cells were cultured in Dulbecco's Modified Essential. These cells were grown at 37 ºC in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 units/mL), and streptomycin sulfate (100 µg/mL) in a humidified incubator with 5% CO_2.

Determination of NO production and the cell viability assay – The amount of NO was calculated by measuring the amount of nitrite, in the cell culture supernatants was perform using Griess reagent as described previously.[10] Briefly, the RAW 264.7 cells (5 × 10^4 cells/mL) were stimulated with or without 0.1 µg/mL of LPS for 24 h in the presence or absence of various concentration of each compounds (1.25 - 100.0 µg/mL). The culture supernatant was used for nitric dioxide determination using Griess reagent.[11] Equal volumes of culture supernatant and Griess reagent were mixed and the absorbance was determined at 570 nm. Cell viability test was performed based on the reduction of using 3-(4,5-demethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St. Louis, MO) reagent into an insoluble, dark purple formazan product in viable cell (5 × 10^4 cell/mL) were incubated with test compounds (1.25 - 100.0 µg/mL) for 24 h. Then, 50 µL of 2 mg/mL MTT reagent was added to the culture plates and further incubated at 37 ºC for 2 h and absorbance was determined at 570 nm.[11]

Result and Discussion

The seven compounds (**1** - **7**) (Fig. 1) were isolated from the methylene chloride and ethyl acetate fractions of the *E. utilis* grains using open column chromatography and HPLC. The structures of these compounds were identified by comparing their spectroscopic data with those in the literature coupled with mass spectroscopic analysis. To the best of our knowledge, compounds **1** - **4**, and **6** were isolated from *E. utilis* grains for the first time.

Compound **1** was obtained as a white solid. The IR spectrum showed typical absorption bands of hydroxyl (3380 cm^{-1}), carbonyl (1672 cm^{-1}), and aromatic substituents (1520 and 1448 cm^{-1}). The ^1H NMR spectrum of **1** displayed *ortho*-coupled A_2B_2-type proton signals at δ_H 7.42 and 6.79 (each 2H, d, J = 8.5 Hz), which were assigned to H-2, 6 and H-3, 5, respectively. In addition, compound **1** showed a pair of *trans*-olefinic proton signals at δ_H 7.58 (1H, d, J = 15.9 Hz) and δ_H 6.26 (1H, d, J = 15.9 Hz). The ^{13}C NMR spectrum of **1** contained one carboxylic acid signal at δ_C 171.2, one oxygenated olefin quaternary carbon signal at δ_C 161.2 (C-4), and six olefin methine carbon signals at δ_C 131.2 (C-2, 6), 116.9 (C-3, 5), 146.7 (C-7), and 115.9 (C-8). Based on the above evidence and comparison with the literature data,[12] compound **1** was identified as *p*-coumaric acid.

Compound **2** was obtained as a white crystalline solid,

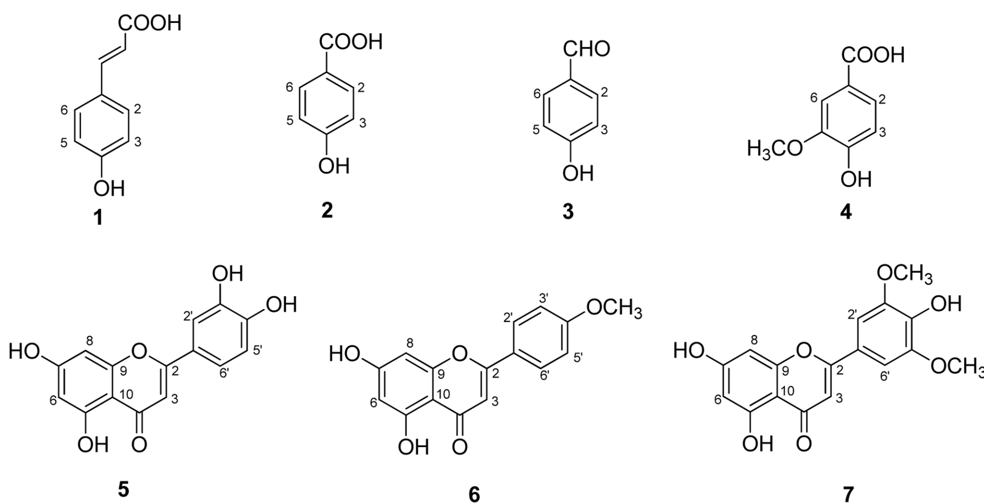

Fig. 1. Chemical structures of compounds **1** - **7** isolated from the grains of *E. utilis*.

and compound **3** was a white solid. The ^{1}H NMR spectrum of **2** showed *ortho*-coupled A_2B_2-type proton signals at δ_H 7.86 and 6.79 (each 2H, d, $J = 8.6$ Hz), which were assigned to H-2, 6 and H-3, 5, respectively. The ^{13}C NMR of **2** contained one carboxyl carbon signal at δ_C 170.5, one oxygenated olefin carbon signal at δ_C 163.7 (C-4), and four olefin methine carbon signals at δ_C 133.4 (C-2, 6) and 116.4 (C-3, 5). The ^{1}H and ^{13}C NMR data of **3** were identical with those of **2**, except for a proton signal at δ_H 9.75 (1H, s) and a carbon signal at δ_C 193.0 (CHO). With the above evidence and comparison with the literature data,[13,14] compounds **2** and **3** were identified as 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde, respectively.

Compound **4** was a colorless gum. The IR spectrum of **4** demonstrated absorption bands for hydroxyl (3384 cm^{-1}), carbonyl (1672 cm^{-1}), and aromatic (1602, 1512 cm^{-1}) groups. The ^{1}H NMR spectrum of **4** displayed an ABX spin system at δ_H 7.54 (2H, overlapped) and 6.82 (1H, d, $J = 6.8$), and one methoxyl proton signal at δ_H 3.87 (3H, s). The ^{13}C NMR of **4** demonstrated one carboxyl carbon signal at δ_C 170.0, two oxygenated olefin quaternary carbon signals at δ_C 152.4 (C-4) and 148.5 (C-5), three olefin methine carbon signals at δ_C 125.1 (C-2), 113.6 (C-3), and 115.6 (C-6), and one methoxyl carbon signal at δ_C 56.2 (OCH_3). Therefore, compound **4** was determined to be vanillic acid by comparing with reported data.[15]

Compound **5** was a yellow powder, and showed IR absorbance bands for hydroxyl (3421 cm^{-1}), carbonyl (1670 cm^{-1}), and aromatic (1621 cm^{-1}) groups. The ^{1}H NMR spectrum of **5** displayed two *meta*-coupling doublets at δ_H 6.21 (1H, d, $J = 2.1$ Hz) and 6.44 (1H, d, $J = 2.1$ Hz), assigned to H-6 and H-8 on ring A of 5,7-dihydroxyflavonoids, respectively. The ABX system at δ_H 7.38 (2H, overlapped) and 6.91 (1H, d, $J = 2.0$ Hz) was assigned to H-5', H-6', and H-2'. In addition, the olefin proton signal at 6.54 (1H, s) was assigned to H-3. The ^{13}C NMR data of **5** showed the presence of a ketone carbonyl at δ_C 184.0, six oxygenated olefin quaternary carbon signals at δ_C 166.5 (C-7), 166.1 (C-2), 159.5 (C-5), 147.2 (C-3'), 151.1 (C-4'), and 163.3 (C-9), and six olefin methine carbon signals at δ_C 114.3 (C-2'), 116.9 (C-5'), 123.8 (C-6'), 104.0 (C-3), 100.2 (C-6), and 95.1 (C-8). Based on the above evidence and comparison with the literature data,[9,15] compound **5** was identified as luteolin.

Compound **6** was a pale yellow powder, and showed IR absorbance bands for hydroxyl (3172 cm^{-1}), conjugated ketone (1660 cm^{-1}), and aromatic (1615 cm^{-1}) groups. In the ^{1}H NMR spectrum, compound **6** contained resonances characteristic for an $ArOCH_3$ group due to a proton signal at δ_H 3.85 (3H, s), the *ortho*-coupled A_2B_2-type aromatic proton at δ_H 7.74 and 7.38 (each 2H, d, $J = 8.0$ Hz) assigned to H-2', 6' and H-3', 5', respectively, and *meta*-coupling proton at δ_H 6.81 and 6.49 (each 1H, d, $J = 2.0$) assigned to H-8 and H-6, respectively. In addition, the singlet proton at $\delta_H = 6.34$ (1H, s) was assigned to H-3. The ^{13}C NMR spectrum displayed 15 carbon signals and one signal from the methoxyl group. These data indicated that **6** belonged to a flavonoid moiety. The carbon signals observed included one ketone signal at 183.8 (C-4), five oxygenated olefin quaternary carbon signals at δ_C 165.8 (C-2), 163.1 (C-5), 166.6 (C-7), 161.0 (C-4'), and 159.6 (C-9), seven olefin methine carbon signals at δ_C 104.3 (C-3), 103.9 (C-6), 95.2 (C-8), 129.6 (C-2', 6'), and 117.2 (C-3', 5'), and one methoxyl carbon signal at δ_C 56.8 (OCH_3). After detailed comparison of the ^{1}H and ^{13}C NMR with those published in literature,[16] compound **6** was identified as acacetin.

Compound **7** was a yellow powder, and showed IR absorbance bands for hydroxyl (3238 cm^{-1}), carbonyl (1661 cm^{-1}), and aromatic (1610, 1506, 1480 cm^{-1}) groups. The ^{1}H NMR spectrum of **7** showed the presence of two *meta*-coupling proton signals at δ_H 6.12 (1H, d, $J = 2.0$ Hz) and 6.39 (1H, d, $J = 2.0$ Hz), assigned to H-6 and H-8, respectively. The methoxyl groups in B ring were detected as two proton singlets in ^{1}H NMR at $\delta_H = 7.16$, attributed to H-2' and H-6' protons. In addition, the singlet proton at $\delta_H = 6.57$ (1H, s) was assigned to H-3. ^{13}C NMR spectrum revealed a ketone signal at δ_C 184.0 (C-4), seven oxygenated olefin quaternary carbon signals at δ_C 166.2 (C-2), 159.6 (C-5), 163.4 (C-7), 166.2 (C-9), 141.3 (C-4'), and 149.8 (C-3', 5'), four olefin methine carbon signals at δ_C 105.5 (C-2', 6'), 100.3 (C-6), and 95.3 (C-8), and two methoxyl signals at δ_C 57.2 (3', 5'-OCH_3). On the basic of the above spectral evidence and comparison with the literature data,[9] compound **7** was concluded to be tricin.

To determine inhibitory activities against LPS-induced NO production of isolated compounds in RAW 264.7 cells, the NO levels were quantified using the Griess reaction. The cytotoxic effect of the isolated compounds was also evaluated in the presence of LPS using MTT assay; the compounds showed no cytotoxicity at the concentration of 100 μg/mL (Table 1). The isolated compounds (**1** - **7**) were examined to determine their inhibitory activities against NO production in LPS-stimulated RAW 264.7 macrophage cells. As shown in Table 1, compounds **5** and **6** displayed half maximal inhibitory concentration (IC_{50}) values of 27.9 ± 2.6 and 14.0 ± 1.1 μM, respectively. Other compounds exhibited weak or no effects (IC_{50}

Table 1. Effects of IC_{50} values of isolated compounds **1** - **7** from *E. utilis* on LPS-stimulated NO production and cell viability.

Compounds	NO production and cell viability[b]	
	IC_{50} value (μM)	Survival rate (%)
1	> 100	> 99
2	> 100	> 99
3	> 100	> 99
4	> 100	100
5	27.9 ± 2.6	> 99
6	14.0 ± 1.1	100
7	> 100	> 99
Dexamethasone[a]	0.4 ± 0.05	100

[a]Dexamethasone was used as a positive control.
Data are present as mean ± SD (n = 3).
IC_{50} values, concentrations inhibiting NO production by 50%.
[b]Nitrite was measured using Griess reaction at 20 h after treatment of LPS (0.1 μg/mL) in the presence or absence of various concentrations (1.25 - 100 μg/mL) of each compounds. Survival rates were carried out at concentration of 100 μg/mL and determine by the MTT colorimetric assay.

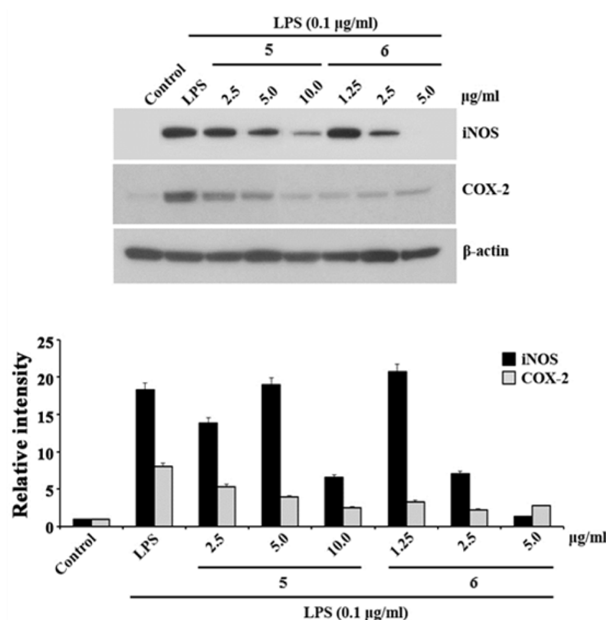

Fig. 2. The effects of compounds **5** and **6** on LPS-induced rise of iNOS and COX-2 levels in RAW 264.7 cells. The RAW 264.7 cells were pretreated with LPS (0.1 μg/mL) for 30 min, followed by concentrations of 2.5, 5.0, and 10.0 μg/mL for **5** and 1.25, 2.5, and 5.0 μg/mL for **6** for 24 h. Whole-cell lysates were blotted with the indicated antibodies. β-Actin level was used as a loading control. The expressions of iNOS and COX-2 were assessed by Western blotting analysis using specific antibodies for individual proteins. The results are representatives of three independent experiments.

values > 100 μM). Compound **6**, a flavonoid with methoxy at C-4' in a B ring displayed the strongest inhibition of NO production in LPS-stimulated RAW 264.7 cells (IC_{50}: 14.0 ± 1.1 μM). Compound **5** exhibited significant inhibitory effects (IC_{50}: 27.9 ± 2.6 μM). The inhibitory effects of active compounds on pro-inflammatory mediators related to the modulation of iNOS and COX-2 expression were tested by Western blotting. The results showed that compounds **5** and **6** (2.5 - 10.0 μg/mL) dose-dependently reduced the LPS-induced iNOS expression (Fig. 2). Therefore, compounds **5** and **6** could suppress LPS-induced iNOS expressions at the transcription level. In addition, pre-incubation of cells with compounds **5** and **6** significantly suppressed the LPS-induced expression of COX-2 protein (Fig. 2). Compound **5** (luteolin), a naturally occurring flavonoid present in many types of plants, was reported to be associated in part with the anti-oxidant potential,[17,18] anti-cancer,[19] anti-inflammatory and anti-allergic activities of LPS.[20] Luteolin was also reported to inhibit NO production and prostaglandin E_2 as well as the expression of iNOS and COX-2.[21,22] The hydroxyl moieties and the double bond between C-2 and C-3 are important structural features in luteolin which are responsible for biochemical and biological activities.[23] Similarly, Compound **6** (acacetin) was also reported to inhibit NO production, the expression of iNOS and COX-2.[24,25]

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